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(54) Title: A DRY POWDER FORMULATION FOR GENE THERAPY

(57) Abstract

Gene therapy using a non-viral vector often requires the administration of large amounts of DNA or RNA dissolved in a solution of relatively small volume. The invention provides a dry powder formulation which contains a lyophilized DNA/liposome complex, a lyophilized RNA/liposome complex, a lyophilized oligonucleotide/liposome complex or a lyophilized protein/liposome complex. The dry powder is suitable for airway delivery or topical administration as an aerosol. The dry powder can be easily reconstituted with water and is active in gene transfer *in vitro* and *in vivo*. The potency of gene transfer of the dry powder was at least 50-fold higher than that of a liquid formulation of similar composition. The composition, method of preparation and method of use are described.

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A DRY POWDER FORMULATION FOR GENE THERAPY

BACKGROUND OF THE INVENTION

Gene therapy is rapidly becoming a new therapeutic modality in experimental and clinical medicine. It is desirable that a pharmaceutical formulation, containing DNA or RNA with the transgene sequence, be prepared in large quantity with pharmaceutical quality. The currently used non-viral vectors for gene therapy are mostly two-vial formulations which are mixed at the bedside prior to administration. Such an admixture formulation is acceptable but not ideal. Furthermore, the two-vial formulations are usually dilute in their concentrations of DNA, RNA, oligonucelotide or protein, necessitating the administration of solutions of relatively large volume. A highly concentrated, stable, one-vial formulation is thus more desirable than the current formulations. We describe here the composition and the method of preparation of a dry powder formulation suitable for topical and airway administration for gene therapy and other purposes. When reconstituted in an aqueous medium, it can also be administered in liquid form.

1. Field of the Invention

The present invention relates to methods and compositions suitable for gene transfer or

delivery of gene therapy. More particularly, it relates to pharmaceutical compositions in which DNA, RNA, oligonucleotides and proteins can be complexed with lipid and delivered in a concentrated and convenient form, either as a lyophilized powder or in liquid form after reconstitution in a suitable aqueous medium.

2. Background Information

The field of gene therapy/gene transfer is currently advancing along a number of fronts, both experimentally and clinically. Genetic material has been transferred using viral vectors, for example adenoviruses and retroviruses, as well as nonviral methods such as microinjection, calcium phosphate coprecipitation, and transfer via liposomes. (For a comprehensive review, see Morgan and Anderson, 1993.) A number of diseases and disorders are potential candidates for some type of gene therapy, if appropriate techniques can be found to deliver genetic material to target tissues and cells. In a few cases, (e.g. Cystic Fibrosis, Familial Hypercholesterolemia and Severe Combined Immunodeficiency) gene therapy has advanced to the point of clinical trials.

Cationic liposomes have been shown to be an efficient means of transfecting a wide variety of mammalian cells. Their advantages include simplicity of preparation, ability to complex a very high percentage of DNA, versatility of use with virtually any type of naked DNA or RNA (with no theoretical size limitation), the ability to transfect many different cell types, superior

transfection efficiency, and lack of immunogenicity or biohazardous activity.

Cationic liposomes composed of a mixture of DC-Chol and DOPE have been used to transfect the airway epithelial cells with CFTR cDNA in CF knock-out mice (Hyde et al., 1993; Alton et al., 1993). The same formulation has also been used in two phase I/II clinical trials for melanoma (Nabel et al., 1992) and for CF (Caplen et al., 1994). This formulation is generally regarded as effective and non-toxic and will become commercially available in the near future.

To date, however, DNA/liposome and RNA/liposome complexes have been administered in liquid form and consequently suffer from several disadvantages. Many are two-vial formulations that must be mixed shortly before administration, resulting in inconvenience in manufacture, shipping, storage and administration. In addition, it is difficult to obtain highly concentrated solutions of DNA and RNA using this technique. This may result in a need to administer large volumes of solution to achieve an optimal therapeutic effect.

SUMMARY OF THE INVENTION

This invention provides a dry powder formulation which contains a lyophilized DNA/liposome complex, suitable for gene transfer or gene therapy. Lyophilized RNA/liposome, oligonucleotide/liposome and protein/liposome complexes are also provided. The dry powder can be easily reconstituted with water and is active in gene transfer *in vitro* and *in vivo*. It is suitable

for airway delivery as an aerosol or for topical administration as a spray. The potency of gene transfer of the dry powder was at least 50-fold higher than that of a liquid formulation of similar composition. In addition, the powder can be reconstituted for delivery in liquid form.

This invention further provides a method of preparing DNA, RNA, oligonucleotides or protein in powder form suitable for therapeutic use or gene transfer.

This invention also provides an effective and efficient method of delivering DNA, RNA, oligonucleotides or protein to tissues or cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. A dry powder formulation of the DNA/liposome complex. 500 μg pUCCMVCAT DNA and 500 nmol (300 μg) of DC-Chol liposomes were mixed and allowed to complex. Lactose was added to a final concentration of 0.25 M. This solution was placed in a -80°C freezer for several hours, further frozen in liquid nitrogen, and freeze-dried for 24 hours. The resulting powder was triturated to reduce the particle size.

Figure 2. Gene transfer activity and toxicity of DNA/liposome complex reconstituted from a dry powder formulation. 2 mg pRSV-LUC DNA and 2 μmol (1.2 mg) of DC-Chol liposomes were mixed and allowed to complex. Dextrose was added to make a 0.25 M solution with a volume of 4 ml. This mixture was freeze-dried and reconstituted with 1 ml of distilled water. This reconstituted complex was

added to CHO cells and allowed to transfect for 44 hours. Luciferase activity (□) and protein content (◆) of the treated cells were assayed. The reconstituted DNA/liposome complex was active in gene transfer at concentrations which showed minimal toxicity, as indicated by the high protein content of the treated cells.

Figure 3. CAT activity in the treated mice. Balb/C mice weighing 20 grams were anesthetized with i.m. injections of ketamine. The dry powder formulation of the DNA-liposome complex (as shown in FIG. 1) was delivered via intra-tracheal administration. The mice were sacrificed after 40 hours and their lungs and trachea were assayed for CAT activity. Each number (1a, 1b) represents a single mouse. Lung samples (for mice 1-8) are noted as "a", while trachea samples are noted as "b". Mice 1 and 2 were instilled with 500 μg of DNA as a fluid reconstituted from dry powder. Mice 3-7 were insufflated with maximally 10μg of DNA as a dry powder. Mouse 8 was an untreated control. Lanes 9 and 10 are a positive control (1 unit CAT) and a negative control (no enzyme) for the CAT reaction, respectively.

DETAILED DESCRIPTION OF THE INVENTION

MATERIALS AND METHODS

The following abbreviations are used in the specification:

DC-Chol: 3ß[N-(N',N'-dimethylaminoethane)-carbamoyl] cholesterol

DOPE: dioleoylphosphatidylethanolamine
CAT: chloramphenicol acetyl transferase
CF: cystic fibrosis
CFTR: cystic fibrosis transmembrane conductance regulator protein
CHO cells: Chinese Hamster ovary cells

Plasmid DNA

Plasmid DNA was prepared by a CsCl/EtBr gradient method (Sambrook et al., 1989). It was run on an agarose gel to test for purity and quantitated by using the A_{260}/A_{280} ratio. Plasmid pUCCMVCAT was constructed in this lab by Dr. Hassan Farhood (*see* Farhood et al., 1995). It contains the *E. coli* chloramphenicol acetyl transferase (CAT) gene driven by the human cytomegalovirus early promoter and cloned into a pUC18 background. Plasmid pRSV-LUC contains a luciferase gene driven by the Rouse Sarcoma Virus promoter (De Wet et al., 1987).

Liposomes

DC-Chol was synthesized by the method of Gao and Huang (1991). DOPE was purchased from Avanti Polar Lipids, Inc. DC-Chol and DOPE were mixed in a 3:2 molar ratio, evaporated in a stream of nitrogen to form a thin film, and vacuum desiccated to remove any residual chloroform. The lipid film was rehydrated in distilled deionized water which had been autoclaved. The liposomes were allowed to hydrate overnight. DC-Chol liposomes were prepared by microfluidization by using an M-110S microfluidizer (Microfluidics Corp.) to an average diameter below 200 nm. The liposomes were filtered

through a 0.2 μm filter to provide sterilization. The liposomes were diluted to a final concentration of 2 μmol/ml (1.2 mg/ml of total lipid). Other lipids known in the art will also be effective for these purposes, with cationic lipids being particularly suitable for complexing with DNA, RNA and other negatively charged compounds.

Examples in addition to DC-Chol include DOTMA, DOSPA, DMRIE, DOTAP, DOGS, and DDAB, which may be used alone or in combination with DOPE or other compounds.

Preparation of DNA/Liposome Complex and Dry Powder

500 μg of DNA and 500 nmol (300 μg) of DC-Chol/DOPE liposomes were mixed and allowed to complex. By complex we mean an association of molecules by noncovalent bonds, such as electrostatic, ionic, hydrophobic, hydrogen bonding and others. Complexation is generally complete in a relatively short time (95% within 10 minutes using the method described here), but will occur at a slower rate in more dilute solutions. This parameter can be determined by routine experimentation by one of ordinary skill in the art, e.g. by changes in light scattering or other methods.

Lactose (or another sugar) was added to a final concentration of 0.25 M. This solution was placed in a -80°C freezer for several hours, further frozen in liquid nitrogen, and allowed to freeze-dry for 24 hours. The resulting powder was triturated to reduce the particle size. The terms "oligo-nucleotide/lipid complex", "DNA/lipid complex",

"RNA/lipid complex", and "protein/lipid complex" refer to the respective compounds that have undergone the steps of complexing and freeze-drying, with or without the addition of a cryoprotective agent. A DNA/lipid complex, for example is a lyophilized DNA/liposome complex.

As will be evident, use of the present method is contemplated for DNA, RNA (both sense and antisense), oligonucleotides, proteins and similar compounds which have all been shown to be deliverable by cationic liposomes (*see*, for example, Felger and Ringold, 1989 (for DNA); Malone et al., 1989 (for RNA); Debs et al., 1990 (for protein); Bennett et al., 1992, (for oligonucleotides). Any cryoprotective agent which can be freeze-dried into powder form (e.g. amino acids, urea) could also be used for the preparation of the powder.

Measurement of Particle Size

Freeze-dried DNA:DC-Chol liposomes were reconstituted to their original volume by addition of deionized water and shaking. The particle size was measured by using a Coulter N4 SD sub-micron particle analyzer, using a uni-modal analysis. The sample was counted for 200 seconds. The particle size of the DNA:DC-Chol liposome complex was between 150-200 nm, both prior to and after the freeze-drying process.

EXAMPLE 1

Transfection of Mammalian Cells In Vitro

2 mg of pRSV-LUC and 2 μmol (1.2 mg) of DC-Chol liposomes were mixed and allowed to complex.

Dextrose was added to make a 0.25 M solution in a volume of 4 ml. This mixture was freeze-dried and reconstituted with 1 ml of distilled water.

The transfection was carried out in a 48 well plate in 0.5 ml as follows. An appropriate amount of the reconstituted complex was diluted into 0.5 ml of serum-free media (composed of 0.25 ml of Hanks Balanced Salt Solution and 0.25 ml of CHO-S-SFM media, both obtained from Gibco BRL), added to CHO cells (obtained from the American Type Culture Collection) and allowed to remain on the cells for 6-7 hours. The serum-free medium was then removed and replaced with 0.5 ml per well of F-12 media (also from Gibco BRL) supplemented with 10% fetal bovine serum (from Hyclone Labs), and the incubation was continued to a total time of 44 hours. For protein measurement, cells were harvested and extracted for protein which was quantitated by using a Coomassie Plus Protein Reagent (Pierce) assay.

Luciferase Assay

CHO cells were allowed to transfect for a total time of 44 hours. The media was aspirated and the cells were washed once with a 0.9% sodium chloride solution. The cells were lysed with 100 µl of Lysis Buffer (Promega) per well. The cell lysate was collected, briefly centrifuged, and 4 µl was used for the luciferase assay. Luciferase Assay Buffer (Promega) was thawed and 10 ml was used to reconstitute the Luciferase Assay Substrate (Promega). The samples were loaded into an AutoLumat LB953 (Berthold) luminometer, 100 µl of the luciferase substrate was added to the cell

lysate, and the relative light units of each sample were counted for 20 seconds. The raw data from each sample was normalized for protein content.

EXAMPLE 2

Airway Delivery of Dry Powder and Reconstituted DNA/Liposome Complex

Balb/C mice weighing 20 grams were anesthetized with i.m. injections of ketamine. A small incision was made into the trachea and a 1 ml Tuberculin syringe with a 21 gauge needle was inserted. The dry powder formulation of DNA:DC-Chol liposome complex was loaded into the syringe. A 1 ml pipet tip with a rubber bulb attached to the top of the pipet tip was attached to the top of the syringe and used to blow the powder into the trachea and lungs. This procedure was repeated several times and a rise in the chest of the mouse indicated a successful insufflation.

In the case of the reconstituted DNA/liposome complex, a small volume (75-100 μl) of distilled water was used to reconstitute 500 μg of the dry powder formulation. A small incision was made in the neck of the mouse to expose the trachea. The entire volume (encompassing 500 μg of the complex) was instilled over a period of one minute.

CAT Assay

The mice were sacrificed 40 hours after insufflation. The lungs and trachea from each mouse were removed, placed in an extraction buffer and homogenized. The sample was briefly centrifuged and

a sample of the supernatant was assayed for protein content.

The CAT assay using [^{14}C] chloramphenicol as a substrate was done according to a modified method of Gorman et al. (1982). A sample of the supernatant was mixed with the assay reaction mixture and incubated for several hours. Ethyl acetate (1 ml) was added to the reaction mixture, vortexed briefly, and 800 μl of the upper layer was taken and dried in a Speedvac for 1 hour. The sample was resuspended with 30 μl of ethyl acetate and then spotted onto a TLC plate. The plate was developed in a chloroform: methanol (95:5) solution and quantified by a phosphorimager.

Effect of Cryoprotectant on the Particle Size and Activity of DNA/Liposome Complex

It is important in some instances, particularly for storage purposes, to include in the formulation of the invention a sufficient amount of a cryoprotectant to permit the formulation to be freeze-dried without loss of activity. Four different sugars (dextrose, sucrose, lactose, and mannitol) were tested for their potential to serve as cryoprotectants. Their ability to maintain the original size of the liposomes and the appearance of the powder mass were used as criteria to select the most appropriate sugar. As shown in Table 1, the original size of the liposomes was 177 nm. Lactose, dextrose, and sucrose were able to maintain the original size of the liposomes, while mannitol was not. Lactose produced a fine white powder upon freeze-drying, while dextrose and sucrose produced a

crystalline substance. Lactose was chosen as the model sugar for the dry powder formulation, although it will be appreciated that other sugars could be usefully employed.

Table 1: Size of Reconstituted Liposomes[a]

Cryoprotectant	Mean Diameter (nm)[b] Cryoprotectant Concentration (M)					
	0	0.1	0.25	0.5	0.75	1.0
Lactose	1450	252	158	128		
Sucrose		171	205	181	156	244
Dextrose		168	273	180	301	397
Mannitol		765	875	985		

[a] Liposomes consist of a 3:2 molar ratio of DC-Chol and DOPE in a concentration of 2 μmol/ml. They were prepared in water and microfluidized to produce liposomes. The mean diameter of freshly prepared liposomes was 177 nm.

[b] The mean diameter was measured by using a Coulter N4SD submicron particle size analyzer using a unimodal analysis. The liposomes were in a volume of 2 ml prior to lyophilization and were subsequently reconstituted with water back to the original volume of 2 ml.

The amount of cryoprotection used in the present formulation can be varied depending on other factors. A suitable amount of the sugar or other cryoprotectant should usually be used to protect the DNA and liposomes during lyophilization. The amount is generally sufficient if the average size of the vesicles is increased or decreased by less than about 50%, and ideally by less than about 20%, as measured by the described method. A cryoprotective substance is considered to be particularly suited to these purposes if, in addition, it produces a powder with bulk and flow properties such that it can be administered as a dry aerosol. Identification of such compounds and suitable amounts or concentrations can be done by routine experimentation by those of ordinary skill in the art.

A Dry Powder Formulation

A dry powder formulation prepared by lyophilizing a 1 ml solution containing 500 μg of DNA, 300 μg of DC-Chol liposomes and 0.25 M lactose is shown in FIG. 1. The powder was fine and flowed very well. It was stored in a desiccator at room temperature until use. Upon reconstitution by adding water, the DNA/liposome complex continued to be 150-200 nm in mean diameter and there was no flocculates or precipitates in the reconstituted solution. The transfection activity of the reconstituted complex was assayed with CHO cells by using complexes containing pRSVLUC DNA. The treated cells were assayed for luciferase activity two days after the

transfection. As shown in FIG. 2, cells could be effectively transfected to express the luciferase activity by the reconstituted complex in a dose dependent manner. Lack of cytotoxicity of the complex was evident because the treated cells contained nearly the same amount of protein as the untreated control (FIG. 2). Thus, we conclude the dry powder formulation, after reconstitution with water, is stable and active in gene transfer for the mammalian cells.

Airway Delivery of the Dry Powder

Dry powder containing pUCCMVCAT plasmid DNA and DC-Chol liposomes was insufflated intra-tracheally into mice. Up to 10 μg of DNA was delivered into each animal. 100 μl of reconstituted complex (500 μg of DNA) from the same dry powder was also intra-tracheally injected as a comparison. The mice were sacrificed two days later and the major organs were removed. CAT activities of protein extracts from trachea and lung tissues of the mice were assayed by using a TLC method. As can be seen in FIG. 3, no CAT activity could be found in either the trachea or lung of the control mouse which had not been injected with any complex (lane 8). Mice treated with dry powders (lanes 3-7) had expressed CAT activity in both trachea and lung tissues. Mice injected with the reconstituted complex also expressed CAT activity in both tissues (lanes 1 and 2). It is important to note that mice treated with the dry powder received only 10 μg of DNA maximally, yet the level of transgene expression

was similar to those treated with 500 μg of DNA as a reconstituted fluid from the same dry powder (FIG. 3). This remarkable and surprising result indicated that the dry powder formulation was at least 50-fold more potent in activity than the reconstituted fluid.

EXAMPLE 3

Treatment of Cystic Fibrosis

DNA which encodes human CFTR (prepared, e.g., as described by Caplen et al., 1994 or Caplen et al., 1995) will be complexed to DC-Chol liposomes, combined with lactose, and lyophilized, as described. The resulting powder will be delivered to the airway of a CF patient using a conventional type of dry powder inhaler (see, e.g., Atkins et al., 1992, Byron, 1990 and Hickey, 1992). In addition to CF, patients with lung cancer and other pulmonary diseases could also be treated using this method with other forms of DNA, RNA, oligonucleotides or proteins. The DNA could contain a tumor suppressor gene for cancer treatment or an antiinflamatory gene for asthma, for example. Since the dry powder is stable it lends itself to being produced in a conventional dosage form. This dosage form is preferred because it would allow patients to self-medicate as each actuation of the inhaler could deliver a predetermined dose. A patient could be treated at home as needed, eliminating the need for hospitalization or medical visits.

DISCUSSION

Since the pioneering work of Felgner et al. (1987), a number of different cationic liposome/micelle formulations have been developed, some of which are commercially available. Our work in this area started with a study of structure-activity relationships in which about 20 different cholesterol derivatives were synthesized and screened for activity and toxicity in a 1:1 mixture with dioleoyl phosphatidylethanolamine (DOPE). A set of empirical rules on the structure of cationic cholesterol was derived for the best transfection activity, best shelf stability, and least cytotoxicity (Farhood et al., 1992). DC-Chol was synthesized according to these rules and determined to be one of the best performing cationic lipids for transfection (Gao and Huang, 1991). It is known that the best transfection activity of cationic liposomes in a serum-free medium occurs at the ratio of positive/negative charge slightly greater than 1 (Gao and Huang, 1991). This is because the complex (with slightly excess positive charge) can readily bind to the negatively charged cell surface. However, most of the optimal DNA/liposome complexes lose a substantial amount of activity when serum proteins are present in the transfection medium. This is the primary reason why the cationic liposomes do not work well if injected i.v., despite some isolated reports of success (Zhu et al., 1993; Brigham et al., 1991). The ratio of DNA to liposome for airway administration varies a great deal among different investigators and ranges from

8:1 (Logan et al., 1995) to 1:8 (Alton et al., 1993) by weight. The role of proteins and other negatively charged molecules in the lining fluid of airway epithelium must play a crucial role in determining the optimal ratio of DNA/liposome for transfection. In the case of solid tumor, we found that 1:0.6 (w/w) ratio of DNA/liposome was optimal if the complex is directly injected into the tumor for transfection (unpublished data). No significant aggregation or precipitation of the DNA/liposome complex occurs at this ratio even if the concentration is greater than 1 mg of DNA per ml. A lyophilized dry powder of the complex could be prepared by using 0.25 M lactose as a cryoprotectant (see FIG. 1). Upon reconstitution, there is no change in particle size (150-200 nm in diameter) and the transfection activity is restored (FIG. 2). *In vivo* gene transfer activity of this powder formulation was shown by intra-tracheal administration into mice. The activity was much greater than that of the reconstituted fluid from the same dry powder.

The dry powder will preferably be administered as an aerosol in a metered dose inhaler. The powder will be suspended in Freon or another suitable agent and packaged into a sealed vial mounted onto an inhaler. Other agents that might be suitable (for example, dichlorotetrafluoroethane and dichlorodifluoro-methane) are known in the art. A unit volume of the Freon suspension will be delivered to the airway by inhalation when the inhaler is triggered to dispense the suspension. Alternatively, the

powder can be loaded into a capsule which is then punctured by an inhaler to release the powder and blown into the airway. Such devices are well known in the medical arts (see, for example, *Pharmaceutical Inhalation Aerosol Technology*, J. Swarbrick, ed., Marcel Decker, New York, 1992, particularly at pp. 155-185 and 255-288; and *Respiratory Drug Delivery*, P.R. Byron, ed., CRC Press, Inc., Boca Raton, Florida, 1990, particularly at pp. 167-206; the entire contents of each are incorporated herein by reference). The dry powder can also be administered topically to skin or mucosal tissues by a similar delivery device.

The DNA/liposome complex can also be reconstituted from the dry powder and used as a liquid in cases where that would be a preferred route of administration. As the powder provides a stable product until reconstitution, any current route of drug administration can be used (oral, intravenous, intramuscular, intraperitoneal, subcutaneous, topical, aerosol, etc.)

While the invention has been described most fully in terms of formulations and uses for gene transfer or therapy, it is not intended to be limited to those uses. It will be clear to the skilled artisan that formulations can also be made from a variety of suitable proteins that might be advantageous to administer diagnostically, therapeutically or experimentally in the manner described. Advantages would include increased stability of the protein/lipid powder over other available forms of the protein, increased efficacy

of delivering either systemic or targeted therapy to the lungs. The invention could be used, for example, in such diverse applications as delivery of chemotherapeutic agents for treatment of lung cancer, or for systemic delivery of insulin in diabetics.

The references earlier mentioned are more fully identified hereafter, and are hereby incorporated by reference and relied upon:

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While the invention has been described in connection with what is presently considered to be the most practical and preferred embodiment, it is to be understood that the invention is not to be limited to the disclosed embodiment, but is intended to cover various modifications included within the spirit and scope of the appended claims.

WHAT IS CLAIMED IS:

1. A dry powder formulation comprising a DNA/lipid complex, RNA/lipid complex or protein/lipid complex suitable for administration to an individual.

2. The formulation of claim 1 where the lipid portion of said complexes comprises 3ß[N-(N',N'-dimethylaminoethane)-carbamoyl] cholesterol.

3. The formulation of claim 1 where the DNA encodes CFTR protein.

4. The formulation of claim 1 where a suitable amount of a cryoprotectant has been added.

5. The formulation of claim 4 where the cryoprotectant is a sugar.

6. The formulation of claim 5 where the sugar is lactose.

7. A method of preparing DNA, RNA, oligonucleotide or protein in powder form suitable for therapeutic administration to an individual, said method comprising mixing said DNA, RNA, oligonucleotide or protein with liposomes in an aqueous solution so that DNA/liposome, RNA/liposome, oligonucleotide/liposome or protein/liposome complexes are formed, and freeze-drying the resulting complexes.

8. The method of claim 7 which further comprises adding a cryprotective agent prior to freeze-drying.

9. A method of delivering DNA, RNA, oligonucleotide or protein to an individual, said method comprising administration of the formulation of claim 1 to the individual.

10. The method of claim 9 where the formulation is administered to the nasal passages, trachea or lungs by means of an aerosol.

11. The method of claim 9 where the formulation is reconstituted in an aqueous solution and administered by nebulization, injection or infusion.

12. The method of claim 9 where the formulation is administered topically.

13. In a method of conducting gene therapy on a mammal in need of such therapy, wherein a DNA or RNA formulation is administered to the mammal, the improvement comprising administering the DNA or RNA as a dry powder formulation which contains a lyophilized DNA/liposome or RNA/liposome complex.

14. The method of claim 13 wherein the dry powder formulation contains a cryoprotective agent.

15. The method of claim 13 wherein the formulation is administered as an aerosol.

16. The formulation of claim 1 wherein said DNA or RNA comprises an oligonucleotide.

17. The formulation of claim 16 where a suitable amount of a cryoprotectant has been added.

18. The formulation of claim 17 where the cryoprotectant is a sugar.

19. The formulation of claim 18 where the sugar is lactose.

FIGURE 1

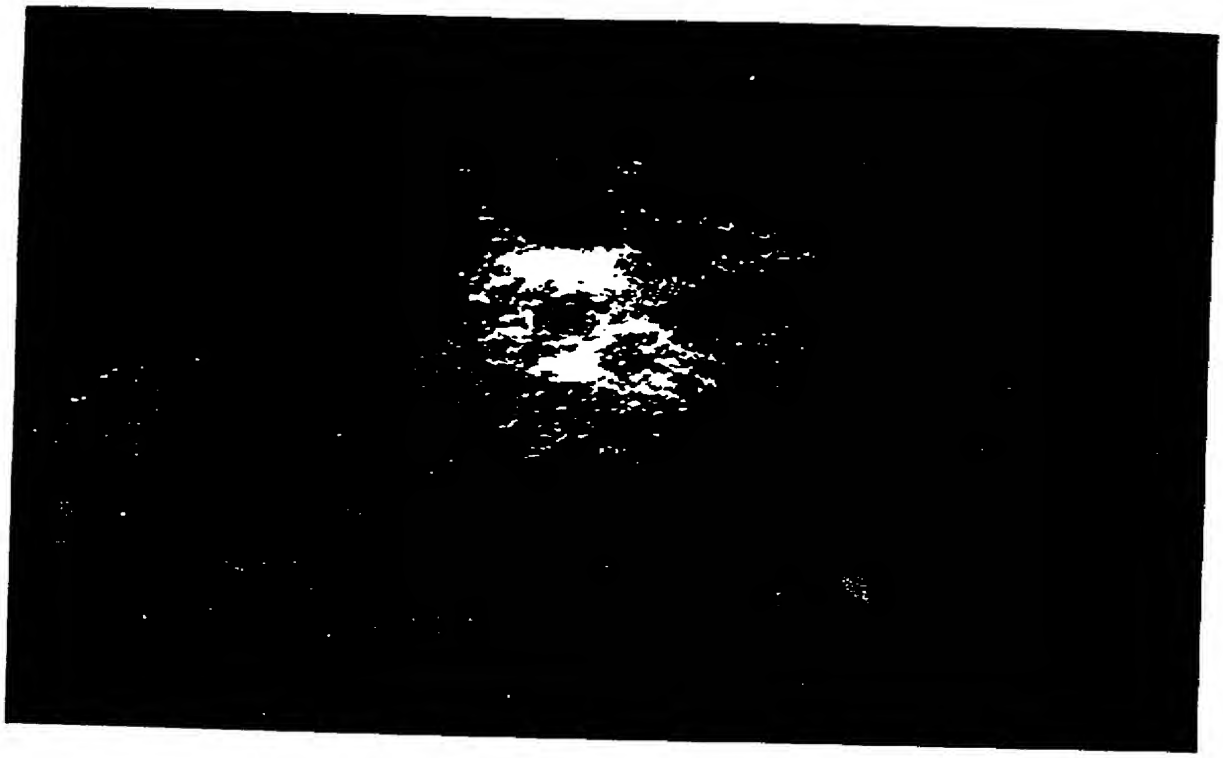


FIGURE 2

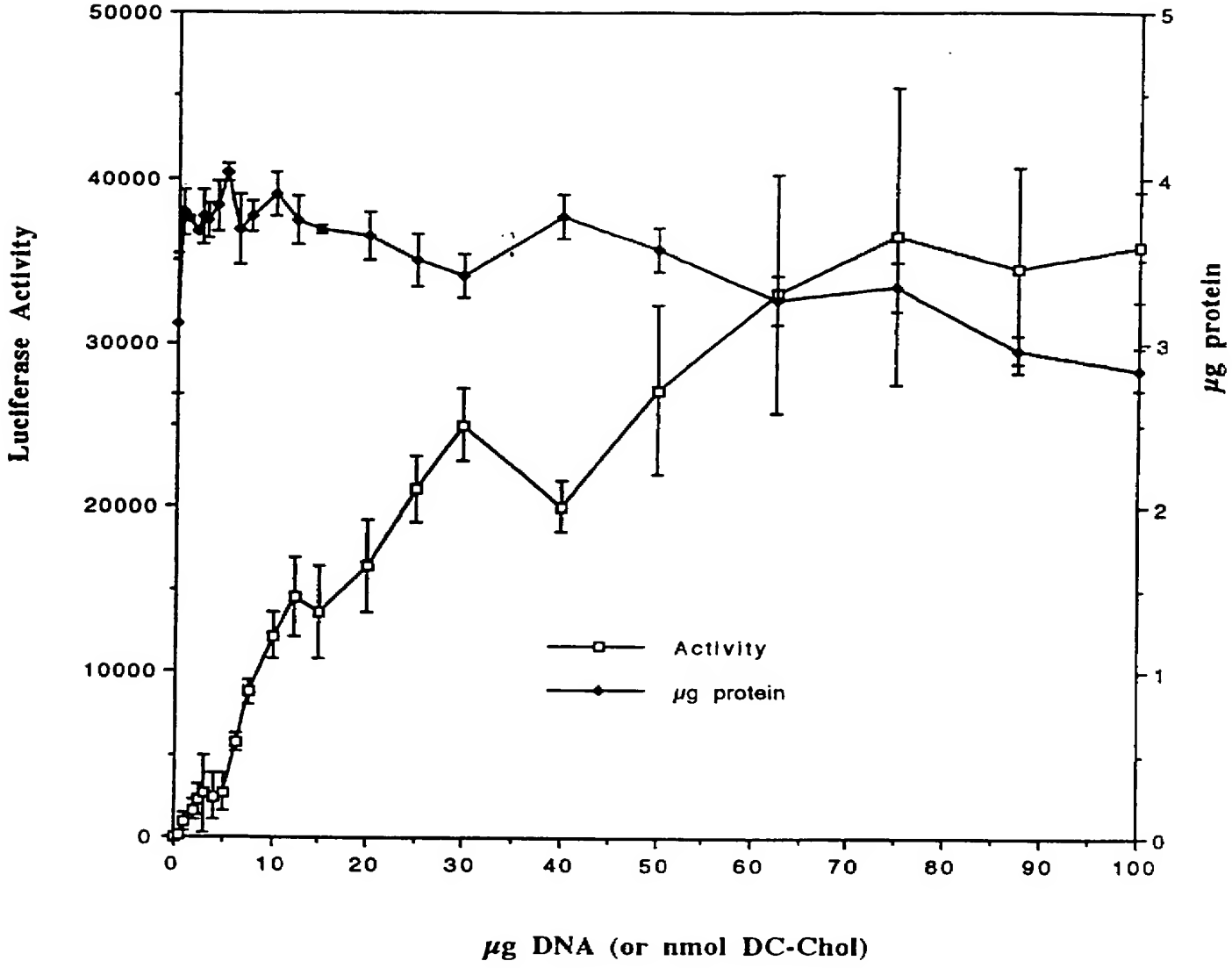
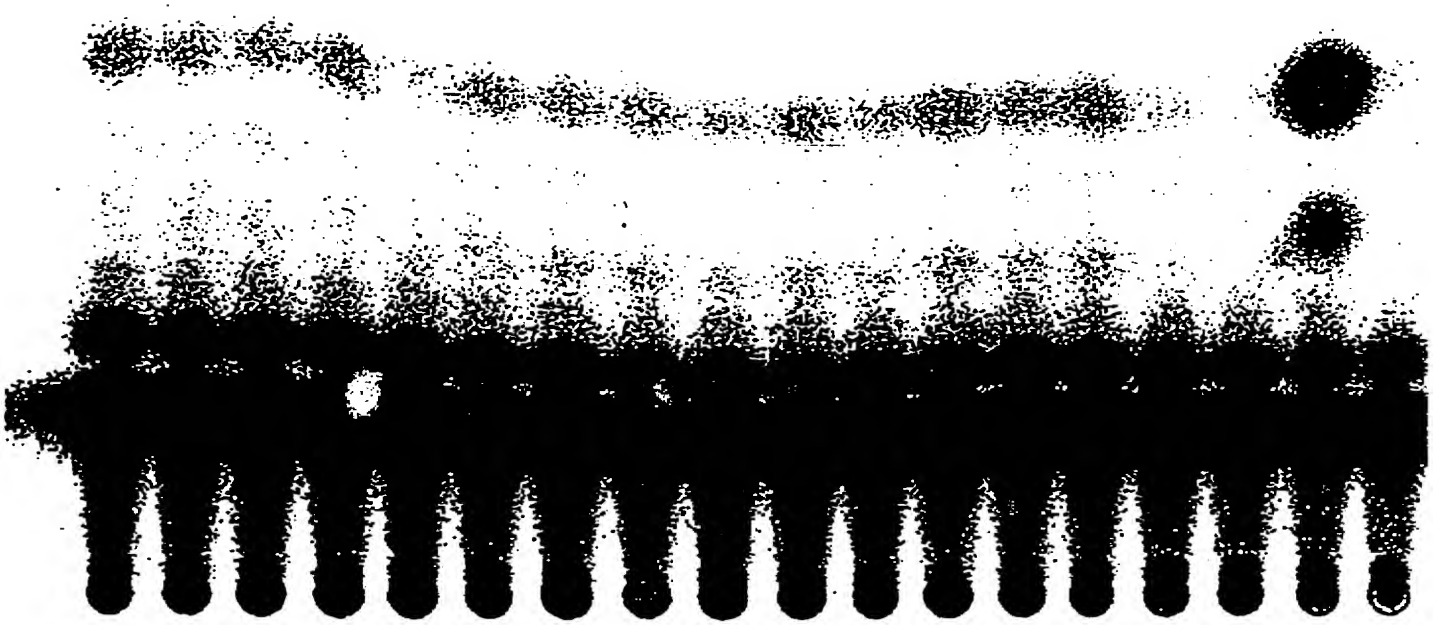

Luciferase Activity
μg protein
μg DNA (or nmol DC-Chol)
Activity
μg protein
50000
40000
30000
20000
10000
0
5
4
3
2
1
0
0
10
20
30
40
50
60
70
80
90
100

FIGURE 3



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02681

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 48/00
US CL :514/44; 435/320.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 435/320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, EMBASE, CAPLUS, WPIDS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,384,128 (MEEZAN ET AL) 24 January 1995, see entire document.	1-19
Y	Chemical and Pharmacology Bulliten, Volume 40, Number 01, issued January 1992, TANAKA ET AL., "Cryoprotective Mechanism of Saccharides on Freeze-Drying of Liposome", pages 1-5, see entire document.	1-19
Y	Biochemical and Biophysical Research Communications, Volume 179, Number 1, issued 30 August 1991, GAO ET AL., "A Novel Cationic Liposome Reagent For Efficient Transfection of Mammalian Cells", pages 280-285, see entire document.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 19 MAY 1996	Date of mailing of the international search report 12 JUN 1996
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